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13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

One hallmark of ovarian cancer is the presence of large amount of floating cells in ascites. However, it is unknown how ovarian cancer cells can survive in absence of adhesion. Our early studies showed that ovarian cancer cells express vitronectin and αvβ3 integrin on cell surface, and the interaction between them is essential for ovarian cancer cell survival in suspension. In present study, we found that engaging αvβ3 with vitronectin induced NF-κB activation; Super electrophoresis mobility shift assay with antibodies against individual member of NF-κB family showed that p50 and p65 were in the DNA-protein complex. To determine the importance of NF-κB activity in ovarian cancer cell survival, we blocked NF-κB activity by either expressing dominant negative form of IκB or using NF-κB inhibitors D609 or SN50. In a 2-day growth period, the inhibition of NF-κB activity resulted in over 80% of cell death in ovarian cancer cells cultured in suspension, but did not significantly affect the survival of ovarian cancer cells cultured in adhesion. These results strongly suggest that vitronectin/αvβ3 interaction-mediated NF-κB activity is essential for ovarian cancer cell survival in absence of adhesion, and may explain why ovarian cancer cells can survive in suspension.

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INTRODUCTION:

The objective of this proposal is to investigate the importance of vitronectin (Vn) and αvβ3 integrin in ovarian cancer. The three main goals of this proposal is to 1) examine the mechanism of Vn/αvβ3-mediated ovarian cancer cell survival in suspension culture; 2) to develop potent hammerhead ribozymes targeted against Vn and the β3 integrin subunit; 3) to suppress the efficacy of adenovirus-delivered Vn and β3 integrin subunit-specific ribozymes to suppress tumorigenicity of human ovarian cancer cells in both *in vitro* and *in vivo* experimental models. We believe the better understanding the role of Vn and αvβ3 integrin in ovarian cancer cell invasion and survival may lead to a novel therapeutic strategy for ovary malignancies.

BODY:

We have accomplished all of first year goals (described as Task 1 in our approved Statement of Work).

In the first part of our study, we have measured the effect of engaging αv integrins with immobilized Vn on NF-κB activity in human ovarian cancer cells. Ovarian cancer OVCAR5 and OVCAR3 cells were transfected with an NF-κB-dependent promoter luciferase reporter gene plasmid, and then added on the culture dishes coated with Vn (2μg/ml) for 2 to 24 hrs. The significant increase in luciferase activity (8-11 fold over the control plasmid-transfected cells) was detected as early as 4 hrs and sustained in all 24 hr period. In a parallel experiment, we also performed electrophoresis mobility shift assay (EMSA). Nuclear extracts were isolated from OVCAR5 and OVCAR3 cells plated on Vn-coated surface for 4 hrs, and then incubated with an oligonucleotide containing NF-κB consensus sequence (Promega) for 30 min. By fractionating the reaction, we found that the mobility of NF-κB consensus sequence-containing oligonucleotide, rather than the mutant NF-κB oligonucleotide, was shifted, further confirming the ability of Vn/αv integrin ligation to induce NF-κB activity. Furthermore, we also performed super EMSA by incubating nucleus/oligonucleotide complex with antibodies to specific member of NF-κB factor. We found that NF-κB members, p65 and p50, were involved in αvβ3 integrin-mediated NF-κB activity. To determine which αv integrin mediates induced NF-κB activity, we pretreated cells with function-blocking mAb to β1 integrin (P4C10), αvβ3 (LM609) or αvβ5 (P1F6) prior to adding cells on Vn-coated surface. We found that Vn-induced NF-κB activity is mediated by αvβ3 integrin rather than αvβ5 or αvβ1 integrin since only function-blocking monoclonal antibody to αvβ3 was capable of abrogating Vn-induced NF-κB activity.

In the second part of our study, we determined the importance of NF-κB activity in ovarian cancer cell survival in suspension culture. We prepared a recombinant adenoviral vector containing dominant negative IκB gene under tetracycline promoter

control (Ad.tet.IκBm) and tetracycline-inducible OVCAR5 cell line (OVCAR5-tet). OVCAR5-tet cells were infected with Ad.tet.IκBm (10pfu/cell) for 24 hrs, and then added in HEMA-coated culture dishes. These cells grew well on HEMA surface (in absence of adhesion); however, the addition of tetracycline, which induced the expression of dominant negative IκB, resulted in over 80% of cell death in a two-day culture period. In a parallel study, we also tested the sensitivity of ovarian cancer cells in suspension to NF-κB inhibitors, D609 (CalBiochem) and SN50 (BIOMOL). Treatment of OVCAR5 and OVCAR3 with D609 and SN50 induced 46.9 and 33.3% of cell death in first day and 81.2 and 77.2% of cell death in second day, respectively. The results from these studies strongly suggest that Vn/αvβ3 ligation-induced NF-κB activity is essential for ovarian cancer cell survival in suspension.

KEY RESEARCH ACCOMPLISHMENTS:

- We found that extracellular matrix vitronectin induced NF-κB activity via αvβ3 integrin in human ovarian cancer cells.
- We found that αvβ3 integrin-mediated NF-κB activity is essential for ovarian cancer cell survival in suspension.

REPORTABLE OUTCOME:

1. Han, Q., New, L., Chen, J., Pan, Z.K., and Huang, S. (2001). Expression of urokinase-specific surface receptor on human carcinoma cells is regulated by interaction with urokinase plasminogen activator. Submitted for *J.Biol.Chem.*

CONCLUSIONS:

One hallmark of ovarian cancer is the presence of adhering cells on peritoneal surface and floating cells in the ascites. However, it is not clear how ovarian cancer cells can survive in absence of adhesion. Our early studies showed that ovarian cancer cells express both vitronectin and αvβ3 integrin on the cell surface, and the interaction between vitronectin and αvβ3 integrin is essential for ovarian cancer cell survial in suspension. In our recent study, we demonstrated that vitronectin and αvβ3 interaction induced NF-κB activation and this induced NF-κB activity is essential for ovarian cancer cell survival. Our present study may explain why ovarian cancer cells can survive in suspension and implicate a novel strategy for ovarian cancer by disrupting or inhibiting vitronectin/αvβ3 integrin expression.

REFERENCES:

None

APPENDICES:

None